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RHIZOPUS ROT OF STRAWBERRIES IN TRANSIT.

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INTRODUCTION.

The increased production of strawberries in the Southern States has resulted in large shipments to northern markets. These shipments have been attended by great losses, due to the decay of the fruit in transit and on the market. In the hope of obtaining information which will assist in reducing this large annual loss, Dr. C. L. Shear and the writers began, in 1915, a study of the organisms and conditions causing decay of strawberries after picking.

This study has included field observations in the principal strawberry-growing regions of Florida, Louisiana, North Carolina, Virginia, Maryland, and Delaware; observations in the markets of Boston, New York, Philadelphia, and Chicago; laboratory studies of the organisms causing strawberry rot; and experimental shipments from points in Florida and Louisiana.[1] These shipments were

[1] Through the cooperation of Mr. George M. Darrow, of the Office of Horticultural and Pomological Investigations of the Bureau of Plant Industry, the writers have received strawberries from points in Alabama, Louisiana, Texas, and other States at times when it was impossible for them to visit these regions. The writers are also indebted to numerous fruit growers and shippers, especially in the central Florida region, for assistance.

NOTE.—This paper is of interest to botanists, pathologists, and strawberry growers and shippers, especially in the Southern States.

made to determine the organisms responsible for the decay of strawberries in transit, the chief sources of infection, how infection could be reduced, and to what extent the growth of organisms could be retarded after they had entered the fruit. With this end in view, the shipments were so handled that every berry could be treated in a definite way and the results recorded after an examination of each berry. This necessitated comparatively small shipments. The writers are of the opinion, however, that the accurate data from these small shipments were more significant in determining the points mentioned than shipments in carload lots with the less accurate methods that must necessarily have been used.

CAUSES OF DECAY IN TRANSIT.

CONDITIONS OF TRANSPORTATION.

Long delay, rough handling, and insufficient refrigeration during transit make it impossible for even the best berries to reach the market in good condition.

Shipments from the extreme south to northern markets sometimes take a week or longer. Even firm, sound fruit, well packed teriorates in value during this period. A delay of a few hours delivery of the berries at their destination may also cause the change from profit to loss on a shipment.

Strawberries usually are bruised unless they are picked too green to be of value for table use. Small containers, such as open crates or pony refrigerators, frequently are subjected to rough handling at points of loading or transfer. When refrigerated cars are used it is necessary to fasten all crates securely in place in order to prevent their shifting during sudden movements of the cars. Bruises to the berries injure their appearance and permit the entrance of decay-producing organisms.

The importance of refrigeration in preserving strawberries during transit has been known for many years. According to Taylor (15, p. 575),[1] it was demonstrated as early as 1868 that strawberries could be shipped from Cobden, Ill., to Buffalo, N. Y., in refrigerator cars maintained at a temperature of 34° to 40° F. The injurious effect of temperatures above 50° F. is clearly shown in the present work (see pp. 7 and 19) and by the experience of shippers in all parts of the country.

CONDITION OF FRUIT.

Strawberries may be in such a condition when shipped that they can not reach the market without great loss, even though rot-producing organisms are not abundantly present. If berries remain in the

[1] Reference is made by number to "Literature cited," p. 21–22.

field until fully ripe, for example, they can not be handled without serious bruising and are soon crushed in shipment.

Varieties differ greatly as to firmness of berry; some can become fully colored before picking and still remain firm for a relatively long time, while others begin to soften before they are colored sufficiently for market. Still others are so delicate when mature that they are bruised by even the most careful commercial handling and are useful only for local markets. Firmness often determines the choice of varieties for commercial production.

The fruit of a variety which ordinarily is a good shipper may under unfavorable conditions lose its firmness. An excess of nitrogenous plant food in the soil is generally believed to induce the growth of soft, succulent berries. Excessive rainfall during the growing and fruiting season has a tendency to produce this effect, especially if accompanied by high temperature. This was noticeable during the shipping season of 1915, especially during May and June, in New Jersey and Delaware, when, within a few hours after berries were picked and before they were placed in cars for shipment, juice would frequently be running from the crates. The fall, winter, and spring of 1915–16, on the other hand, were unusually dry, especially in the extreme south, and it was remarked by growers, shippers, and retail merchants that berries shipped from Florida to northern markets in 1916 reached their destination in better condition than in any previous year.

Severe losses sometimes result from hot weather prevailing during transit or while the fruit is being picked and loaded for shipment. Since this damage is due to the more rapid growth of fungi at the higher temperature it will be discussed later (p. 7).

The shipping qualities of berries may be injured seriously by heavy storms. On February 24, 1916, a rather severe hailstorm occurred at Lakeland, Fla., and for several days its effect on the berries was noticeable; bruises developed into soft spots as the fruit ripened, though the epidermis often was not broken by the hail. In some fields nearly all the berries were bruised, and it was impracticable to prevent the packing of some injured fruit. On March 22, 1916, a very strong wind blew for several hours over all sections of Florida from which strawberries were being shipped. Sand and fine dust were driven by the wind with such force that for several days strawberry plants were coated with dust and the fruit had a dull, dirty appearance. Berries picked during the days immediately following this storm were extremely soft and did not ship well. Severe rainstorms also cause much damage to berries, both by mechanical bruising and by covering them with dirt, which often is difficult to remove.

These and other unfavorable field conditions may result in the production of berries which even with careful handling and thorough

refrigeration can not be transported to distant markets without loss. Although berries may be in the best of condition when picked, they often decay before reaching the consumer, owing largely to the activity of rot-producing organisms.

FUNGI.

Bacterial rots are rare in strawberries. This may be due to the high acid content of the fruit. On the other hand, ripe strawberries, especially if injured, furnish a favorable substratum for the growth of many fungi. Among the less important fungi found were species of Alternaria, Botrytis, Cladosporium, Dematium, Fusarium, Mucor, Patellina, and Penicillium. All these fungi will grow on strawberry tissue, and under unusually favorable conditions some of them may cause appreciable damage.

Botrytis, long known to occur commonly on strawberries and frequently found in fruit from some sections, is not of first importance as a cause of decay in transit. Berries affected by Botrytis in the field are recognized readily and should be thrown out as culls. The growth of Botrytis in the ripe strawberry is so slow that but little damage occurs in the short time between picking and marketing.

Penicillium, which occurs frequently in small quantity, has been abundant in only one case which has come under the observation of the writers. In a shipment from Florida in the spring of 1916 in which the berries were very soft, owing apparently to the effect of the severe sand storm previously mentioned, Penicillium was very common. It is uncertain whether this was due to the condition of the berries or to some unusual opportunity for infection.

By far the most important cause of rot of strawberries in transit is a species of Rhizopus. Not only is this fungus the most common one on strawberries from every section studied by the writers, but in some instances it is almost the only fungus present. During the spring of 1916 the writers examined more than 14,000 decayed strawberries shipped on various dates from three localities in Florida. Most of these were kept until quite rotten, and more than 90 per cent of them developed only Rhizopus.

In addition, several hundred inoculations on strawberries of various varieties have been made, and in no case has Rhizopus failed to produce typical rot when introduced into a wound (fig. 1). No similar results could be obtained with any of the other fungi found.

RHIZOPUS NIGRICANS.

In his recent monograph of the genus Rhizopus, Hanzawa (8) used physiological as well as morphological characters in distinguishing the species. The writers have not investigated the physiology of this fungus sufficiently to compare with Hanzawa's results, but so far as

the morphological characters are concerned the fungus under consideration when grown under certain conditions agrees very well with his description of *Rhizopus nigricans* Ehrb. It is evident from his description that Hanzawa studied the fungus under rather uniform conditions, as the measurements given by no means include its limits of variability.

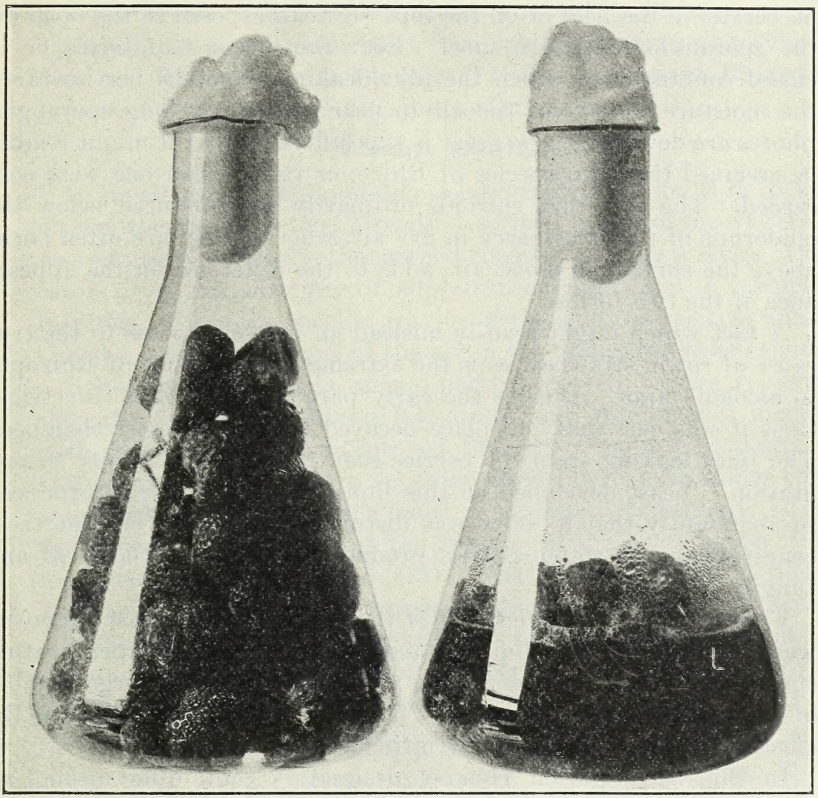

FIG. 1.—Effect of *Rhizopus nigricans* on strawberries of the Missionary variety which had stood for two days at room temperature. Each berry in the flask at the right had been inoculated with *Rhizopus nigricans*.

In connection with studies on the humidity relations of this fungus (p. 7), the writers found that at a temperature of 20° C. the length of the sporangiophore and the size of the columella vary greatly in air of different humidities. For example, in air with a saturation deficit of 17 mm. of mercury, 2.3 per cent relative humidity, sporangiophores are mostly 1 mm. in length, while in saturated air they are from 3 to 4 cm. long. In air with a saturation deficit of 17 mm. of mercury the columellæ are mostly 95 to 100 μ in width; in saturated air they are mostly from 150 to 180 μ wide. The size of spores and

the diameter of hyphæ do not seem to vary greatly with the different humidities. The hyphæ range from 10 to 40 μ in thickness, mostly 20 to 30 μ. The spores are variable in shape and size, being round to oval, or even very irregular, 5 to 15 μ in diameter, the large irregular spores often reaching a length of 27 to 28 μ.

The extreme length of the sporangiophore is apparently developed only in saturated or nearly saturated air. When the fungus fruits on berries in the field or on the tops of boxes exposed to the open air the sporangiophores are short. Near the bottoms of boxes or in closed containers in which the juice leaking from the berries raises the moisture content of the air to near saturation, long sporangiophores are developed. So great is this difference that it might readily be assumed that two species of Rhizopus rather than one were concerned. The fact that rhizoids ordinarily are produced below the epidermis of the strawberry in dry air, whereas they are often borne above the surface in moist air, adds to the difference in the appearance of the two forms.

A fact which might readily mislead an investigator as to the true cause of rot in strawberries is the extreme susceptibility of Rhizopus to alcohol vapor. During the early part of the writers' investigations it was customary to place decayed berries in moist chambers. The juice leaking from the berries soon raised the humidity to saturation. Yeast developed on this liquid, and alcohol was produced so abundantly that its odor was distinctly noticeable. Under these conditions many rotten berries produced no fruiting bodies of any fungus.

Finally it was observed that if the covers of the moist chambers were removed and the liquid taken up with blotting paper, mature sporangia of *Rhizopus nigricans* would develop on a large number of the rotten berries overnight. This led to an investigation of the effect of alcohol fumes on the fruiting of this fungus.

In this investigation Hempel desiccators containing alcohol of various strengths were used (200 c. c. of alcohol solution per desiccator). It was found that berries inoculated with Rhizopus would rot under 95 per cent alcohol without producing any fruiting bodies. The same thing proved true of all strengths of alcohol down to 2 per cent. With 2 per cent alcohol aerial mycelium developed, but no sporangia were produced; with 1½ per cent alcohol apparently normal sporangia on short sporangiophores developed.

In 1896 Goff (6) found that strawberries exposed to alcohol vapor under a bell jar in a refrigerator did not mold, while similar berries kept in the same refrigerator but not exposed to alcohol vapor molded considerably; he makes no mention, however, of the species of fungi present.

PHYSIOLOGY OF RHIZOPUS NIGRICANS.

CHARACTER OF THE ROT PRODUCED.

The rot produced in strawberries by *Rhizopus nigricans* differs from that caused by any other organism the writers have observed and has been briefly described in an earlier paper (13). Its chief characteristic is the rapid collapse of the berry, with the loss of much juice. So great is this loss that the juice often drips from boxes or crates containing strawberries rotted by Rhizopus, and this has given rise to the name "leak" applied to this type of rot by shippers and dealers. F. L. Stevens (12) has recognized Rhizopus as the cause of this type of rot in strawberries.

HUMIDITY RELATIONS.

In investigating the humidity relations of this fungus in its attack on strawberries, a method already described by one of the writers (14) was employed. Briefly, the method consists in using a series of desiccators filled with mixtures of sulphuric acid and water in various proportions. From the specific gravity of the acid the relative humidity and the saturation deficit of the air in the desiccator were calculated.

Repeated experiments, using partly decayed berries received in shipment, as well as sound Klondikes and Missionaries inoculated with *Rhizopus nigricans*, proved that the fungus will destroy the strawberry as quickly in a very dry atmosphere, even under concentrated sulphuric acid, as in a moist or saturated atmosphere. Certain differences in the attack of the fungus on the berry under various humidities have been noted (13). The important fact in this connection is that when the fungus is once inside the strawberry no amount of external drying is sufficient to stop its development. The moisture contained in the fruit will permit the fungus to grow sufficiently to destroy the berry.

TEMPERATURE RELATIONS.

The temperature relations of *Rhizopus nigricans* have recently been studied by two investigators. Hanzawa (8) studied the temperature relations of various species of Rhizopus and used these relations as criteria for separating the genus into sections. He places *Rhizopus nigricans* in the section characterized by having no growth at 37° C. (99° F.). He found that this species was able to grow and produce sporangia at temperatures as low as 8° to 10° C. (46° to 50° F.).

Miss Ames (1) included *Rhizopus nigricans* among the storage-rot fungi whose temperature relations she investigated. She found that at 9° to 10° C. (48° to 50° F.) some growth occurred, although at this temperature the fungus was unable to mature sporangia within one month. Maximum growth occurred at 36° C. (97° F.), good growth at 37° and 40° C. (99° and 104° F.), and no growth whatever

at 42° C. (108° F.) Miss Ames also studied the time required for the germination of spores at various temperatures and found that in general the length of time required for germination decreased with rise of temperature, being greatest, 200 hours, at 3° to 4° C., and least, 5½ hours, at 41° C.; no germination occurred at 42° C. The difference in maximum temperatures as determined by these investigators may be due either to their having different strains of the fungus or possibly to their having used different culture media. Miss Ames used Rhizopus from sweet potato grown on string-bean infusion; Hanzawa's temperature tests were made with the fungus growing on potato.

In the writers' experiments on the temperature relations of *Rhizopus nigricans* the fungus was grown on ripe strawberries and on white corn meal in flasks. The refrigeration plant used by Drs. Brooks and Cooley, of the Bureau of Plant Industry, in their studies of the storage rots of apples was used. This gives satisfactory constant temperatures at intervals of 5 degrees from 0° to 20° C. In addition, for other low temperatures use was made of an ice thermostat, and for the higher temperatures thermostats heated by gas burners were used.

Sound berries of the Missionary variety shipped from Plant City in commercial refrigerators were inoculated with spores and mycelium of Rhizopus and placed at temperatures of 0°, 5°, 10°, 15°, and 20° C. At the end of five days berries kept at 0° and 5° showed no growth of the fungus and were as sound and fresh in appearance and taste as when first placed in the refrigerator. At 10° C. there was no aerial growth of the fungus and only a very tiny decayed spot about the region where inoculation was made. Most of the berry at this temperature was still perfect in appearance and taste. Berries at 15° and 20° C. bore abundant fruiting hyphæ and were typical "leaks." "Leak" is a term applied to fruit destroyed by Rhizopus because of the collapse of the berries and the leakage of juice from the boxes. Cultures in corn-meal flasks at the same temperatures showed in five days no growth at 0°, 5°, or 10° C., but abundant mycelium with sporangia at 15° and 20°. At 15°, however, there was only about half as much aerial growth as at 20° and about half the sporangia were white and immature. At 20° the surface of the flask was covered with mature black sporangia. Similar flasks kept for five days at 1°, 2°, 4°, 6°, 8°, 11°, 14°, and 18° C. showed no growth up to 8°, slight growth with occasional sporangia at 11°, and fairly numerous sporangia at 14° C.

Berries inoculated as described were kept for two weeks at 0°, 5°, and 10° C. Those kept at 0° and 5° were apparently as sound as when placed in the refrigerator. At 10° the berries were somewhat softened, and there was a slight aerial growth of mycelium near the

point of inoculation. Several boxes of commercial berries kept at 10° C. for one week showed about the same proportion of decayed berries as similar boxes kept at room temperature for 12 hours. Very little work was done on the investigation of temperatures above 20° C. The fungus appears, however, to be able to mature a few sporangia with short stalks on ripe strawberries at 36° to 37° C. From these experiments it appears that a temperature of 10° C. (50° F.), or, better, 8° C. (46° to 47° F.), is satisfactory for preserving strawberries from this fungus for a week or more. The rate of growth of this fungus increases so rapidly with rise of temperature above this point that the importance of keeping the berries at this temperature is obvious.

In this connection it is of interest to note that Earle (4) in his shipping experiments found that 50° F. was satisfactory for shipping strawberries.

HOST RELATIONS.

From microscopic study (13) it is apparent that *Rhizopus nigricans* is unable to penetrate the sound epidermis of the strawberry. Additional evidence as to the correctness of this view is found in the results of experiments in which wounded and unwounded berries were sprinkled with spores of Rhizopus. The wounded berries in all cases rotted quickly and bore typical sporangia, while the unwounded berries remained uninfected.

Inoculation experiments in field and laboratory show that Rhizopus is able to grow in green strawberries and produce typical leak if the epidermis is wounded. On February 21, 1916, green berries shipped from Florida were wounded and inoculated with Rhizopus. Two days later all these berries had developed mature sporangia. Ten similar berries, unwounded, over which spores of Rhizopus were sprinkled showed no change. While still on the vines 100 green berries were wounded and inoculated with Rhizopus mycelium from a pure culture. All of these developed the typical Rhizopus rot, most of them before maturity.

OCCURRENCE ON OTHER PLANTS.

Rhizopus nigricans has been noted frequently as the cause of rot in various vegetables. Ehrenberg (5), in connection with his original description of the genus, mentions its occurrence on decaying fruits of *Cornus masculus*, *Morus alba*, and once on apple (*Pyrus malus*). Behrens (2) found it causing rot of tomatoes (*Lycopersicon esculentum*) and was able to produce decay by inoculation in pears (Pyrus), plums (Prunus), raspberries (Rubus), and currants (Ribes). Wormald (17) and Hanzawa (8) report it as causing a decay of tomatoes. In this country it is probably best known as causing a rot of sweet potatoes (Ipomoea), although Halsted (7)

found it to be the most important cause of decay of quince (Cydonia) fruit, Orton (11) reports it as causing a soft rot of white potatoes (Solanum), and Hawkins (9) corroborates the observations of Orton. The writers have also found this species on red raspberries in the Washington market.

This fungus may at times cause a serious rot of pears, and to some extent of peaches, as shown by the following manuscript note furnished by Mr. M. B. Waite:

In September, 1902, shipments of Kieffer pears from Patuxent (Woodwardville P. O.), Md., to the Washington, D. C., market suffered very severely from decay induced by *Rhizopus nigricans*. Pears of this variety had been shipped with success from 4-year-old trees in 1900 and from 5-year-old trees in 1901 by picking them hard and green, beginning about September 10 and continuing through the month. After picking they were stored in bins, usually for about a week, partly covered with canvas cloth. The layer of pears, about 2 to 2½ feet thick, colored up nicely without shriveling and brought a good price on the market.

Shipments started in this way in 1902, picked about September 10 and shipped a week later, began to arrive in good condition. Daily shipments were made, and a few days after the first arrived trouble was experienced with a soft rot, found on examination to be due to *Rhizopus nigricans*. The individual fruits attacked were completely disintegrated, those not attacked remaining sound.

About September 20, a carload of fruit was shipped in barrels and baskets. The barrels were of the open-head truck type, with cloth covers. The baskets were of the ordinary slatted, 4/8, 25-pound Delaware type. About half the fruit in baskets was salable at a reduced price, some of the baskets being one-third or one-half rotted.

The fruit in barrels, however, was almost a total loss. About 50 or 60 of these barrels were placed on arrival in the second story of a commission house, to allow them a few days to ripen, and the juice leaked freely out of the more open barrels through the floor and dropped down to the lower story. The tighter barrels became slightly swelled, so that they were water-tight, and on examination they were found to be partly filled with the liquefied contents, with a few perfectly sound pears floating or immersed in the liquid. The fruit was packed on one day, delivered to the city by rail on the afternoon of the following day, and hauled to the commission house. On the following morning the rot was found starting in strongly, and in another 24 hours the results already described appeared.

The fruit was thought to be fairly well handled by methods that had given good results in previous years, but the Kieffer pear is very hard and brittle, and the epidermis is easily ruptured by rubbing or scratching. The inability of the fungus to penetrate certain fruits is accounted for by their complete freedom from the slightest injury to the cuticle. Later pickings from the same orchard, with cooler weather and perhaps somewhat better handling, gave good results. Fruit picked and shipped green the first week in October stood the trip to Washington and was immediately placed in cold storage without any traces of Rhizopus rot. Shipments were made for 10 years following this without encountering this difficulty to any appreciable extent.

Occasionally trouble somewhat similar has been experienced to a minor degree in shipments of peaches. Last summer (1915), about August 15, a refrigerator carload of Belle peaches, shipped to the Washington market from Clarks Gap, Va., showed this type of rot before it was completely sold. The car opened up in good condition, and part of the fruit was sold the morning after

arrival in good order. Some of the remainder was held in the car two or three days and part of it at a commission house, and it deteriorated very rapidly, owing to the attacks of *Rhizopus nigricans*. The fruit leaked badly, many of the peaches becoming completely penetrated and overgrown by the rot fungus. In this case the fungus appeared to grow readily from one fruit to another, possibly through the stem end.

It is significant that in all cases where the rot caused by *Rhizopus nigricans* is described it is characterized by the loss of a large quantity of liquid from the decayed tissue. Behrens (2) and Wormald (17) speak of this in discussing the rot of tomatoes, Halsted (7) in quince, Orton (11) in potatoes, and Waite in pears and peaches.

The observations recorded above show that Rhizopus is the chief and in some sections practically the only cause of rot of strawberries in transit. They show that the percentage of moisture in the air makes no difference in the rate of growth of the fungus inside the berry. Also, that while the growth of Rhizopus is very slow at 10° C. (50° F.) or below, the rate increases rapidly with the rise of temperature above that point, so that at favorable temperatures a strawberry infected with Rhizopus is destroyed in a few hours. Microscopic studies by one of the writers (13), as well as inoculation experiments and field observations, indicate that Rhizopus does not usually penetrate the uninjured epidermis of the strawberry.

From this work it is apparent that no method of drying the air of the container in which the strawberries are shipped, by ventilation or otherwise, will retard the decay of the fruit once the fungus has entered, but that rot of strawberries in transit may be diminished greatly by reducing the amount of infection by Rhizopus or by keeping the berries at a low temperature from the time they are picked until sold. The importance of low temperature in shipping strawberries was demonstrated by Parker Earle during the period from 1866 to 1872 (3 and 4). Earle found that strawberries cooled to 50° F. in a specially constructed cooling house previous to shipment might be sent long distances in refrigerator cars in practically perfect condition.

PRESENT SHIPPING PRACTICES.[1]

To appreciate the general lack of complete refrigeration and the importance of reducing infection by Rhizopus, it is necessary to know something of the methods of handling strawberries now in general use.

[1] Descriptions of the development of refrigerator transportation of truck produce were published in 1901 by F. S. Earle (3) and W. A. Taylor (15). Of particular interest in this connection are the descriptions (3, p. 444; 15, p. 575) of the early work of Parker Earle in shipping strawberries under refrigeration. So far as the writers have been able to learn, Parker Earle designed the first refrigerator chest for shipping strawberries by express and was the first to demonstrate the effectiveness of precooling. His letter (3, p. 444–445) places the beginning of this work about 1866, and it was mentioned by Holcomb in 1870 (10).

In most localities the berries are either shipped in the containers in which they are picked, without further handling, or emptied on tables, the culls removed, and the sound fruit repacked for shipment. The former method necessitates great care in picking; the latter method undoubtedly bruises the berries somewhat even when carefully done.

Practically all berries shipped from Florida are packed in quart cups, each cup "capped" or "plated"; that is, the top layer consisting of berries of uniform size placed snugly together with their tips all pointing in the same direction. Shipments from this State are made at a season when no other berries are in the markets and are intended to supply a demand for fancy fruit.

The strawberries are shipped in three ways. During the early part of the season most of them are shipped by express in iced containers, called pony refrigerators, which hold 64, 80, or occasionally 32 quarts each. From 75 to 250 pounds of ice is placed in an open pan in each refrigerator and the refrigerator tightly closed. In this manner berries are often shipped long distances, to New York, Boston, Philadelphia, Pittsburgh, Chicago, and other northern markets. Later in the season iced cars are shipped from the more important strawberry-growing centers, but the use of the pony refrigerator is at no time abandoned. Under favorable weather conditions many shipments from Florida to Washington, Baltimore, and other relatively near-by markets are made by express in open ventilated crates without refrigeration. The use of different methods of shipment increases the difficulty of controlling merely by refrigeration the growth of Rhizopus during transit and emphasizes the necessity of preventing the infection of the fruit. Experiments were performed to determine the relative importance of the various possible sources of infection.

In many strawberry-growing sections it is customary to mulch with hay, straw, pine needles, or other material to protect the fruit from dirt. In other localities this practice is not followed, and when the fruit is picked immediately after a rain or when wet with dew much sand or other dirt adheres to the berries. This fruit is sometimes shipped to market without cleaning, but usually an attempt is made to avoid or correct this condition. In some localities picking the berries early in the morning when wet with dew is avoided, and the dry sand is shaken off in the process of picking. In other places the fruit is emptied on tables covered with cloth and shaken gently, most of the sand being lost in this way. In central Florida, a region important for the earliness and length of its shipping season, it is customary to wash the berries when brought from the field. They are immersed for a few seconds in a tub of water, then either packed moist or spread on a cloth frame

and allowed to dry in the shade of the packing house or even in the sun.

SOURCES OF INFECTION BY RHIZOPUS NIGRICANS.

INFECTION IN THE FIELD.

Infection with Rhizopus apparently could occur either in the field before picking or in the packing shed. Microscopic examination of diseased fruits (13), as well as observations and inoculation experiments in the laboratory, the field, and the packing house, indicates that Rhizopus rarely, if ever, enters berries through the uninjured epidermis. The proportion of injured or wounded berries in a field usually is low. Some fruits, of course, are injured by insects or birds, but rapidly growing strawberries have a strong power of preventing infection by the quick covering of wounds with an impervious protective layer.

This was well illustrated in an inoculation experiment made in the field on February 23, 1916. One hundred sound berries of various ages from one to seven days before maturity were wounded with a sterile needle while still on the vines. One hundred similar berries were wounded in the same way, and a small quantity of dirt from underneath the plants was forced into each wound. One hundred wounded berries in another series were inoculated with mycelium from a pure culture of Rhizopus. These berries were picked when mature and their condition compared with that of 100 uninjured berries. No rot was apparent among the fruits merely wounded. All but two of the berries into which dirt had been introduced matured without showing any signs of infection; a callus layer underneath the wound was evident in each case. Two exceptions showed at the end of five days soft spots on one side of the wounds. All of the berries inoculated with Rhizopus mycelium developed into typical leaks in from two to four days, most of them before the maturity of the berry. Leaky berries are rarely, though occasionally, found in the field.

Similar experiments were performed with ripe berries at the time of picking and the berries shipped in iced containers to Washington. In this series some of the fruits were wounded also with pine needles from the mulch. Records taken after shipment showed the following percentages of fruit still sound: Unwounded, 72 per cent; wounded with sterile needle, 61 per cent; wounded with pine needles, 62 per cent; dirt introduced into wounds, 42 per cent. Those inoculated with Rhizopus mycelium had become a mass of rotten berries before reaching destination. Wounding the fruit with pine needles gave practically the same result as the use of a sterile needle. The introduction of soil into the wounds apparently induced con-

siderable decay, but none of the berries so treated developed into typical leaks within 48 hours after removal from the refrigerated container, and the presence of black dirt in the wounds was misleading and made the accurate sorting of these berries difficult.

The above experiments indicate that the fungus in question is not of primary importance as a field rot, that usually it does not enter the berries from the soil, and that in the localities investigated infection occurs at some time after picking.

UNSANITARY CONDITIONS USUALLY PREVAILING IN THE PACKING HOUSE.

Conditions in and around the packing house often are unsanitary, favoring the growth and distribution of Rhizopus, and the frequent handling and consequent bruising which the berries often undergo would seem to offer many chances for infection. The culls often are thrown on the ground near the packing house; various fungi, including Rhizopus, may be found fruiting on these discarded berries. Packing tables become soaked with strawberry juice and usually are not cleaned or disinfected during an entire season. When berries are washed, a few gallons of water are placed in a tub and used for all berries cleaned during the day. Fungus spores, soil, and other dirt soon accumulate in this wash water, and apparently might by this means be distributed generally over a large proportion of the fruit.

Conditions in packing houses and cars as regards infection from Rhizopus can be easily improved by the frequent use of hot water. The recent work of Thom and Ayers (16) shows that the spores of this fungus are easily killed by heat when wet, but are more resistant in dry air. Rhizopus spores (16, p. 159) were killed in 30 seconds at a temperature of 145° F. in milk, but survived a temperature of 200° F. when dry. It is probable, however, that even with the greatest care all sources of fungous growth and distribution can not be eliminated.

THE BRUISING OF BERRIES IN PACKING.

It has been shown that Rhizopus rarely, if ever, enters berries through the unbroken epidermis; care to avoid bruising is therefore of great importance. This fact was emphasized by an experiment in which sound berries were wrapped individually in tissue paper, carefully packed, and shipped to Washington. It was found that fruit so treated could be held at room temperature for a week or more after arrival without the appearance of decay, while similar berries packed and shipped in the commercial manner were badly rotted within 48 hours after reaching their destination.

Berries are injured to some extent every time they are handled or shaken. When possible, especially when shipments are made in car lots under refrigeration, it is preferable to ship in the containers in

which the fruit is picked without further handling. However, fruit shipped in crates without refrigeration or in pony refrigerators, as from Florida, usually suffers severely by careless handling at points of transfer. In these cases the close packing of the fruit to prevent settling has been found to be of much advantage. If packed too tightly or forced into place, the fruit is unnecessarily crushed and bruised. The aim of the best packers is to place the berries as closely as possible without crowding or injuring them.

DIRECT SOURCES OF INFECTION.

One experiment was conducted in which the berries were treated in various ways to obtain, if possible, some indication as to the most serious sources of infection with Rhizopus in commercial handling. Apparently sound berries were selected, and in order to accentuate the comparative value of the results obtained the epidermis of each berry (except in A and C, Table I) was broken with a sterile needle before other treatment. All berries were then packed similarly and shipped in small iced containers. The results of the experiment are given in Table I.

TABLE I.—*Infection of strawberries resulting from various methods of treatment.*

Treatment of fruit, if any.	Condition after arrival at destination (per cent).		
	Sound.	Soft spots.	Leaks.
A.—Sound fruit, not wounded	72	28	0
B.—Wounded with sterile needle	61	32	7
C.—Wounded with pine needles	62	35	3
D.—Washed in clean water	66	22	12
E.—Washed in water used in packing house	0	71	29
F.—Dirt forced into the wounds	42	58	0
G.—Rhizopus mycelium forced into wounds	0	0	100
H.—Washed in water containing spores from a pure culture of Rhizopus	0	0	100

Table I shows that injury with pine needles and washing in clean water did not injure the shipping qualities of the berries any more than did injury with the sterile needle. The contrast between the final condition of fruit washed in clean running water and that washed in the ordinary commercial manner was most striking. This preliminary shipment gave a valuable clue, which was followed in subsequent experiments. In all later shipments sound, unwounded berries selected individually by one of the writers or those selected by careful professional packers were used.

WASHING BERRIES.

The influence of washing upon the development of rot in strawberries was investigated. In each of a series of shipments made in small iced containers, commercial pony refrigerators, or ventilated

crates without refrigeration, some of the berries were washed in the tubs of water which had been previously used for the washing of several bushels of commercial berries, some were washed in clean running water, and the remainder were not washed. All washed berries were dried for a few minutes in the shade. They were then packed by professional packers (all berries in each shipment being packed by one operator) and sent to Washington, where records were taken upon a careful inspection of each berry. The results of these experiments are summarized in Table II.

TABLE II.—*Infection resulting from washing strawberries in a commercial manner.*

Treatment of fruit, if any.	Number of berries.	Sound after arrival.
		Per cent.
Not washed	716	37
Washed in clean water	1,153	35
Washed in dirty water used in the packing house	1,067	17

The generally low average of sound fruit was due to the length of time the berries had been kept at room temperature after reaching their destination. In northern markets, however, one frequently finds berries which contain no higher proportion of sound fruit. The results given in Table II indicated that the commercial method of washing berries was injurious to their shipping qualities and also that this injury would be largely overcome by the use of clean water. The frequent changing of water in the tub usually would not be inconvenient in Florida, for wells and pumps often are located at or near the packing houses.

DRYING BERRIES AFTER WASHING.

As already mentioned, a few growers make a practice of packing their fruit wet, though most of them expose it to the air, either in the shade of the packing house or, more frequently, in the sun, until most of the water has evaporated from the surfaces of the berries. Experiments were undertaken to determine the relative effects of these different methods from the standpoint of inhibiting the growth of Rhizopus in the fruit.

When berries are dried they are spread upon cloth-covered frames or packing tables. These cloth covers usually are not renewed during a shipping season. Often they are soaked with juice from the strawberries, and it was suspected that they might serve as carriers of infection to fruit spread upon them. Numerous careful experiments for the determination of this point, however, gave

negative results, indicating that any increase of infection from this source is relatively unimportant.

In investigating the effect of drying the fruit after washing, a number of experimental shipments were made, the fruit being handled in the same manner as in the washing experiment already described except that part of the berries in each shipment were packed wet, while the remainder were partially dried, usually by exposure to direct sunlight for a few minutes. The results were conclusively in favor of packing the fruit wet. Taking the average of all these experiments, it was found that drying berries washed in clean water had reduced the proportion of sound fruit 36 per cent. Among those washed in dirty water the reduction caused by drying was even greater—43 per cent.[1] Table III summarizes the results of all experiments on drying the berries after washing.

TABLE III.—*Effect of drying strawberries after washing.*

Treatment of fruit, if any.	Packed without drying.		Packed after drying.	
	Number of berries.	Sound after arrival.	Number of berries.	Sound after arrival.
		Per cent.		*Per cent.*
Not washed	1,939	50		
Washed in clean water	2,886	48	2,881	30
Washed in water used in the packing house	650	33	1,144	19

In no case were these results reversed, though they differed in degree in the various shipments. These variations were correlated with differences in temperature when the berries were packed, the comparative benefit of wet packing being greater in those shipments prepared on very warm days than in those packed during cool, cloudy weather. Drying the fruit, even in the shade, proved injurious, though less so than exposure to direct sunlight.

The laboratory experiments already cited indicate that the rate of growth of *Rhizopus nigricans* increases very rapidly with the rise of temperature above 10° C. (50° F.). When infected berries are held for a few hours at a relatively high temperature, for instance, 30° C.

[1] Smith and Goodman, of the Department of Agriculture of British Columbia, have conducted experiments upon the fanning of strawberries before shipment. They report (Winslow, R. M. Report of horticultural branch, 1914. *In* 8th/9th Rpts. Dept. Agr. [Brit. Col.], 1913/14, p. 84. 1915.) that berries dried for an hour in a strong current of air from an electric fan carry to market in better condition than those shipped even slightly wet. Though not so mentioned in their report, this operation would have the effect of lowering the temperature of the berries quickly and considerably by increasing the rate of evaporation of the water from their surfaces. It would thus be, in effect, a precooling process, allowing the berries to be placed in the car at a relatively low temperature if loaded at once; if, after fanning, however, the fruit were hauled several miles to the station during hot weather, much of the benefit of rapid drying would be lost.

(86° F.), the fungus develops so rapidly in their tissues as to cause them to collapse. The growth of the fungus may be checked by lowering the temperature, but with ordinary refrigeration growth will not entirely cease, and it may continue sufficiently to cause the berries to soften in transit; it will in any case resume rapid development whenever the temperature is again raised, as when the berries reach their point of destination. If infection has occurred, a difference of a few degrees in the temperature of the berries, maintained for several hours, will make a decided difference in the length of time before rot becomes apparent. This accounts for the harmful effects of drying the berries.

Dry berries remain at air temperature until placed in iced cars or other refrigerated containers, usually a period of several hours. During part of this time the crates are often exposed to the sun or covered with a dark cloth. When berries are immersed for a few seconds in water their temperature is lowered somewhat. Subsequent evaporation from their surfaces results in still further cooling. If they are packed immediately after washing, evaporation continues slowly and exerts a cooling effect for several hours, usually until the berries are placed under refrigeration. When berries are being loaded into cars in the afternoon, several hours after picking, the difference in temperature between unwashed fruit and that packed wet can be readily felt with the hand. When, after washing, the berries are dried for a few minutes in the shade, the water evaporates before packing, and they lose the continued cooling effect of prolonged evaporation. If they are exposed to direct sunlight there is the added disadvantage of a further rise in temperature. The beneficial effect of packing the berries wet is greatest, of course, when the temperature of the air is highest.

Professional packers claim that berries are softened by washing and must be handled with great care to avoid bruising if packed while wet. This probably is true, but it is more than compensated for by the cooling of the fruit.

Exposure to sunlight, unless very brief, has the further undesirable effect of giving the berries a dull appearance. Heating unwashed berries by exposure to the sun increases rot, but not so markedly as with washed berries. This is illustrated by an experimental shipment prepared at Lakeland, Fla., on the morning of March 22, 1916, a clear, warm day. Sound berries of the Missionary variety were used. After treatment they were packed by a professional packer, carried to the railroad station at noon, and shipped to Washington in a ventilated crate without refrigeration. They were three days in transit. Their condition on arrival is shown in Table IV.

TABLE IV.—*Effect of drying or heating strawberries.*

Treatment of fruit, if any.	Unwashed fruit.		Washed fruit.	
	Number of berries.	Sound after arrival.	Number of berries.	Sound after arrival.
		Per cent.		*Per cent.*
Packed without drying or heating	396	72	352	69
Exposed to air (21° C., 70° F.) in shade of packing house	430	64	.....	.....
Exposed to sunlight (30° C., 86° F.)	412	56	416	47
Spread on table covered with black cloth and placed in sun; temperature 36° C. (97° F.)	473	37	.....	.....

Observations at points of shipment and destination show that car lots of berries picked and shipped on very warm days under similar conditions of transit do not arrive at northern markets in such good condition as those shipped during cooler weather. This further illustrates the fact that the temperature of the berries from the time they are picked until placed under refrigeration influences their shipping qualities.

A final series of experiments was undertaken to substantiate further the previous results in regard to the effect of washing fruit in clean water and drying before packing. In this series, as before, shipments were made in small iced containers, commercial refrigerators, and in ventilated crates without refrigeration. Table V summarizes the results of these shipments. They agree with the former experiments in showing the harmful effects of drying and emphasize the benefit derived from packing the fruit wet, for berries treated in this manner arrived at their destination in even better condition than those not washed.

TABLE V.—*Effect of washing strawberries in clean water and of subsequent drying.*

Treatment of fruit, if any.	Number of berries.	Sound after arrival.
		Per cent.
Not washed	3,027	46
Washed in clean water and packed wet	2,809	51
Washed in clean water and dried before packing	2,826	32

SUMMARY.

The conclusions given here are drawn chiefly from field studies and experiments made in Florida and Washington in the winter and spring of 1916 and in Louisiana and Chicago in 1917.

The deterioration of strawberries in transit may be due to the condition of the berries at the time of shipment or to inadequate trans-

portation or refrigeration facilities, but it is often induced by fungi, chiefly *Rhizopus nigricans.*

Rhizopus causes the softening and rapid collapse of strawberries, accompanied by the escape of much juice.

Berries though bearing the spores of Rhizopus when shipped may still, with proper handling and refrigeration in transit, reach the market in good condition. The fungus is not able to penetrate uninjured epidermis, but enters readily through wounds in either mature or green berries.

Rhizopus has not been found of primary importance as a cause of field rot, but usually develops in berries at some time after they are picked.

The humidity of the air has no perceptible influence upon the rate at which *Rhizopus nigricans* rots strawberries.

The growth of *Rhizopus nigricans* is very slow at 10° C. (50° F.) or below, but increases rapidly with the rise of temperature above that point. Therefore the proper cooling and refrigeration of the berries from the time they are picked until marketed will reduce the losses from this rot.

Since the diversity of shipping practices does not always permit such complete refrigeration, the reduction of infection by means of sanitation and careful handling is important.

The conditions at the packing houses are frequently unsanitary, affording favorable opportunities for the growth of Rhizopus, and bruising the fruit during handling allows infection to take place. The packing houses and tables should be kept clean. Injury to the fruit should be avoided by care in handling, or preferably, by the elimination of all unnecessary handling.

Washing berries in water in which dirt has accumulated injures their shipping qualities.

The temperature of the berries during the time between picking and shipping has an important influence upon the later development of rot.

Drying washed berries, especially by exposure to sunlight, increases decay, because the subsequent exposure of the dry berries to prevailing high air temperatures favors the development of Rhizopus within their tissues. The higher shipping quality of fruit packed wet seems to be due to the facts that the temperature of the fruit is lowered by washing in cold water and that by prolonged evaporation the berries are kept cool for a considerable time, during which they may be hauled to the station and placed under refrigeration.

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